REMARKS

Applicants have amended the claims as shown above. The amendments incorporate the description of terms in the specification and place the claims in better form for appeal, notice of which is filed concurrently herewith.

Applicants have amended Claims 1 and 51 to expressly state that the step of immobilizing nucleic acids on one side of a non-siliceous surface (Claim 1) or of a membrane (Claim 51) occurs in the presence of an immobilization buffer, that the immobilization buffer comprises any one of several enumerated compositions, and that the immobilized nucleic acids are released by applying to the non-siliceous surface or membrane an elution agent that is either water or a salt solution. In addition, Applicants have amended Claims 1 and 51 to expressly include the proviso that the nucleic acids that are subsequently released from the non-siliceous surface (Claim 1) or membrane (Claim 51) do not contact any solution that has contacted the opposing side of the surface or membrane on which the nucleic acids were not immobilized. Support for these amendments to Claims 1 and 51 is found throughout the specification and claims. By way of example of such support, see, p. 5, lines 4-14; p. 6, line 29 ("Suitable salts for the immobilization of nucleic acids . . . ") - p. 8, line 9; p. 9, line 26 ("Suitable eluting agents for the elution of bound nucleic acids . . .)" - p. 10, line 12; p. 11, lines 6-13; Examples 1-3; Example 8 and Table 6; Example 9 and Table 7; Example 10 and Table 8; Example 11 and Table 9; Example 12 and Table 10; Example 13; Example 15 (elution salts study); Claims 10, 13, 18, 22, and 70-74. Accordingly, the amendments reiterate the description of the invention provided by the original disclosure and claims and, therefore, add no new matter.

Entry of the amendments to the claims is respectfully requested.

Response to Rejection Under 35 USC § 112, second paragraph

In the Office Action, the Examiner objected to the term "immobilizing" as indefinite in Claims 1-5, 9-22, 24-41, 44-51, 53-55, 58-64, and 67-75 under 35 USC § 112, second paragraph. In particular, the Examiner stated:

> "It is unclear and indefinite what this [term] means. For example the nucleic acids can be actually bound to the surface (e.g. membrane) or the nucleic acids can be entrapped or retained on the surface (e.g. through ultracentrifugation [sic]). As written the claim is ambiguous thus making the metes and bounds of the invention indefinite." (p. 3 of the Office Action, Paper No. 20)

Applicants respectfully traverse the rejection for the reasons provided below.

The claims are read and understood in view of the description of the invention provided by the specification. Applicants' specification provides an extensive description of a process of preferentially isolating nucleic acids from even very complex mixtures of various molecular species (e.g., cell lysates). The specification clearly teaches that in one step of the process nucleic acids are bound to an immobile phase (see, in the specification, e.g., p. 3, line 29-p. 4, line 1) using an immobilization buffer (see, e.g., p. 6, line 29-p. 8, line 9, of the specification). Multiple examples compare various buffers for use in immobilizing nucleic acids to a non-siliceous surface or membrane according to the invention (see, e.g., Examples 1-3; Example 8 and Table 6; Example 9 and Table 7; Example 10 and Table 8; Example 11 and Table 9; Example 12 and Table 10; and Example 13, of the specification). Such immobilization buffers may also be used as optional washing buffers to wash the immobilized nucleic acid without releasing or losing the immobilized nucleic acids (see, e.g., p. 9, lines 23-24 of the specification). Moreover, the specification makes clear that release (elution) of the immobilized nucleic acids from a surface is subsequently effected by applying a distinctly different type of solution, i.e., an elution agent, such as water or an aqueous salt solution (see, e.g., p. 9, line 26-p. 10, line 12, of the specification). Thus, persons skilled in this art who have the benefit of Applicants' specification would understand that the steps of immobilizing and releasing nucleic acids are clearly controlled by the presence of different *solutions*. Clearly, the term and context for "immobilizing" nucleic acids (and not proteins or all types of molecules) excludes other means of separating molecules such as ultrafiltration that uses a surface that retains *any* type of molecule above a certain size or a material that has a high binding affinity for one type of molecule over others. Provided a person skilled in the art is able to follow the claimed process according to the specification, Applicants are not required to also provide the exact *mechanism* that occurs when nucleic acids are immobilized to one side of a non-siliceous surface or membrane while proteins and other molecules pass through to the other side.

The above comments show that Applicants' specification provides a person skilled in this art with a thorough description and context for understanding the term "immobilizing" in the context of the claimed invention. Nevertheless, to reiterate the teachings of the disclosure in the claims, Applicants have also amended independent Claims 1 and 51, as noted above, to expressly

state that the step of immobilizing nucleic acids to one side of a non-siliceous surface (Claim 1) or of a membrane (Claim 51) is carried out in the presence of an immobilization buffer and, further, that such an immobilization buffer comprises any of a number of key ingredients as described in the specification and as recited in dependent Claims 10, 13, 18, 22, 26, and 70-74.

In view of the above comments and amendments to the claims, Applicants submit that the term "immobilizing" as recited in the claims is clear and definite under 35 USC 112, second paragraph. Accordingly, Applicants respectfully request that the Examiner reconsider the objection and reverse the rejection of the claims.

Response to Rejection Under 35 USC § 102: the Mullis Reference

The Examiner rejected Claims 1-5, 9-14, 18-21, 24-26, 28-41, 44, 50, and 59-63 as anticipated by US Patent No. 5,234,824 ("Mullis"). Applicants respectfully traverse the rejection for the reasons provided below.

As noted previously in this record, Mullis teaches a method of isolating DNA from blood cells that requires gentle lysis of blood cells and avoidance of high shear forces to permit sufficiently high molecular weight DNA to be released and trapped in certain cellulose acetate filters (membranes). According to the method of Mullis, a filter containing DNA is then physically transferred to a vessel and immersed and shaken (e.g., on a rotary shaker) in an elution buffer at an elevated temperature (e.g., 37°C-100°C). If the buffer evaporates, the filter may be rehydrated with fresh buffer. After immersion and shaking, the elution buffer containing the eluted DNA is then removed (see, e.g., col. 6, lines 55-68; Example 3, col. 9, lines 55-64; Example 6, col. 12, lines 28-36, of Mullis). In contrast to Mullis, Applicants' claimed invention is not restricted to the isolation of DNA molecules that are released from lysed blood cells and that are sufficiently large to be trapped in the pores of certain cellulose acetate filter membranes. Indeed, the specification demonstrates that nucleic acids of varying sizes and compositions (e.g., RNA versus genomic DNA) are readily isolated by Applicants' invention (see, e.g., Examples 5 and 6, of the specification).

Moreover, in Mullis, the step of releasing the entrapped DNA by immersing the membrane in an elution buffer necessarily destroys the distinction between where the released DNA is relative to each side of the membrane. In Mullis the released DNA contacts elution buffer that contacts both sides of the membrane. In contrast, according to Applicants' claimed

invention, the physical distinction of where the eluted nucleic acid is relative to each side of a non-siliceous surface (Claim 1) or membrane (Claim 52) is *maintained throughout the process*. According to Applicants' invention, a non-siliceous surface or membrane is charged with nucleic acids that are immobilized on one side of the surface or membrane, and the nucleic acids are subsequently released and retrieved from the same side on which they were immobilized. In particular, during the step of releasing (eluting) the immobilized nucleic acids, the eluted nucleic acids never contact any solution that has passed through to and contacted the opposing, "waste" side of the non-siliceous surface or membrane on which the nucleic acids were not originally immobilized (see, e.g., p. 11, lines 6-13; Examples 1-14, of the specification).

However, in the Office Action, the Examiner stated that Applicants' previously argued distinctions between the method described by Mullis and the method of Applicants' invention referred to "limitations and embodiments that are simply not contained in the claims" (p. 5 of the Office Action). Although Applicants believe that persons skilled in this art who have the benefit of Applicants' disclosure would readily distinguish the claimed invention from the method of Mullis, Applicants have nevertheless amended Claims 1 and 51, as noted above, to expressly recite such aspects of the invention. Thus, Claims 1 and 51, as amended herein, expressly state that the step of immobilizing the nucleic acids on one side of a non-siliceous surface (Claim 1) or membrane (Claim 5) is carried out in the presence of an immobilization buffer that, in the case of Claim 1, comprises any of the limitations of original Claims 10, 13, 18, and 22, and, in the case of Claim 51, comprises any of the limitations of Claims 70-73. Applicants also have amended Claims 1 and 51 to expressly state that the step of releasing the immobilized nucleic acids from one side of the non-siliceous surface or membrane is carried out by applying an elution agent that is water or an aqueous salt solution as taught in the specification (see, e.g., p. 9, line 26-p. 10, line 10; Example 3; Example 15 and Table 12, of the specification). Furthermore, Claims 1 and 51, as amended herein, now expressly recite the proviso that the released nucleic acids do not contact any solution that has contacted the opposing side of the non-siliceous surface or membrane on which the nucleic acids were not immobilized. This proviso particularly and accurately reiterates a feature of Applicants' process and sufficiently distinguishes the claimed invention from the process in Mullis.

In view of the above comments and the amendments to the claims, Applicants submit that it is clear that Mullis does not teach or suggest each and every step of Applicants' claimed process for isolating nucleic acids. Accordingly, Mullis does not anticipate Applicants' claims, and Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

Response to Rejection Under 35 USC § 102: the Ogawa Document

The Examiner rejected Claims 1-5, 9-14, 24-26, 28-30, 32, 39, 44, 50, 59-60, and 62 as anticipated by European Publication No. 0 431 905 ("Ogawa"). Applicants respectfully traverse the rejection for the reasons given below.

Ogawa describes a method for purifying bacteriophage DNA, e.g., from M13 phage particles, which method has three basic steps and is dependent on the use of at least one ultrafiltration membrane (ultrafilter) in the final step.

> 1. Bacterial cells and debris are separated from mature phage particles by passing a culture medium through a membrane filter having pore size of 0.22-0.45 micrometers. Persons skilled in this art recognize this standard culture filtration step to routinely separate bacteriophage released into culture medium from bacterial cells or large cellular fragments.
>
> 2. The phage retained on the membrane (filtrate from step 1.) are then subjected to one or more agents to denature and/or decompose the phage protein coat to liberate phage DNA, e.g., by applying to the phage particles an organic solvent, detergent, protease, or alkali treatment (col. 3, lines 1-27, of Ogawa).
>
> 3. The mixture of denatured or decomposed viral protein and liberated phage DNA is applied (if not already) to an ultrafilter membrane that is suppose to retain molecules of the liberated phage DNA from molecules of the size of the denatured or decomposed proteins. (see, col. 2, line 31-col. 4, line 9; col. 4, lines 20-37, of Ogawa).

Persons skilled in this art understand that an ultrafiltration membrane, like gel filtration column chromatography, retains a certain size range of molecules but cannot preferentially separate types of molecules from one another (e.g., nucleic acids, proteins, carbohydrates, lipids, detergent micelles, organelles). Ogawa specifically requires and relies on the use of an ultrafiltration membrane in the final step (or sooner if introduced at step 2) that can retain molecules that have the size of the liberated phage DNA (which must be known), but permit

molecules of the size of the denatured or decomposed viral proteins to pass through the ultrafilter. Ogawa also states that if the molecular weight of the DNA is larger than the fractionation molecular weight of the ultrafilter, the DNA may also *pass through* this ultrafilter (col. 3, lines 31-37, of Ogawa). Clearly, a process based on a critical ultrafiltration step requires confidence and knowledge of the various sizes, kinds, and relative abundance of various molecular species present in a sample in order to select the appropriate ultrafilter.

Unlike the method of Ogawa, Applicants' claimed process for isolating nucleic acids is not based on and does not recite a step that employs an ultrafiltration membrane to indiscriminately retain a certain range of molecular sizes. Unlike Ogawa's method, Applicants' claimed process does not recite a step in which protein is retained and denatured in the presence of nucleic acids retained on an ultrafiltration membrane. In contrast, Applicants' claimed method is clearly and expressly controlled by the presence of particular *solutions* and the *preferential immobilization of nucleic acids to one side of a non-siliceous surface or membrane.* In Applicants' invention nucleic acids are preferentially immobilized on one side of a non-siliceous surface or membrane in the presence of an immobilization buffer while simultaneously other contaminating molecules, e.g., proteins, carbohydrates, etc., simply pass through to the opposing, waste side of the non-siliceous surface or membrane. Applicants' claimed process is carried out using a non-siliceous surface or membrane without requiring any knowledge necessary to select an appropriate ultrafiltration membrane such as the size of the nucleic acids of interest or other contaminating molecules present in the original sample.

Note also that Ogawa teaches that alkali treatment may be used to denature the protein. However, persons skilled in this art know that the use of such alkali conditions to denature proteins as taught in Ogawa would also destroy RNA (see, e.g., "Hydrolysis of Nucleic Acids by Acids and Bases", *In* Lehninger, A., Biochemistry, second ed. (Worth Publishers, Inc., New York, 1975), pp. 322-323 (attachment A)). Thus, in contrast to Applicants' claimed invention, the method of Ogawa also cannot be used to isolate RNA.

The above comments clearly show that Ogawa describes a specific method for purifying phage DNA, and particularly bacteriophage M13 DNA, that employs a selected ultrafiltration membrane and a step to denature phage protein retained on that ultrafiltration membrane. Such steps are not recited in Applicants' claims. As Ogawa does not teach or even suggest each and

every step of Applicants' claimed process for isolating nucleic acids, Ogawa does not anticipate Applicants' claims under 35 USC § 102. Accordingly, the Examiner is respectfully requested to reconsider and withdraw the rejection.

Response to Rejection Under 35 USC § 102: the Schneider Document

The Examiner rejected Claims 1-5, 9-14, 24-26, 28-30, 32, 39, 44, 50, 59-60, and 62 under 35 USC § 102 as anticipated by European Publication No. 0 442 026 ("Schneider"). Applicants respectfully traverse the rejection for the reasons described below.

Schneider describes a method of isolating genomic DNA from human blood cells that depends on extraction of protein with an organic solvent and the subsequent formation and trapping of micellar cationic detergent-genomic DNA complexes. Schneider provides a detailed protocol that includes the following "main" steps:

1. human blood is lysed with a cationic detergent,

2. extraction of proteins with an organic solvent (e.g., chloroform),

3. centrifugation to eliminate a protein clog at the organic phase,

4. diluting the aqueous phase with water to decrease the ionic strength and adding a cationic detergent so that the resulting cationic detergent micellar-DNA complex precipitate after a short mixing operation,

5. ultrafiltration of a solution containing the DNA-cationic micellar complexes to retain the complexes from solution,

6. washing the retained DNA-cationic micellar complexes with a low ionic strength solution to eliminate excess detergent, then washing with a mixture of alcohol and NaCl to change the DNA-cationic detergent complex into DNA-Na, then washing with a mixture of alcohol and low ionic strength solution to remove excess salts, then resuspending the genomic DNA in water or a dilute salt solution. (see, col. 2, line 2-col. 3, line 9, of Schneider)

The method of Schneider is clearly a process specifically designed for isolating genomic DNA that relies on a step-by-step chemistry that is completely different from that employed in Applicants' claimed invention. Applicants' claimed invention is recited in the claims, as

amended herein. As noted above, Applicants' claimed method comprises preferentially immobilizing nucleic acids, even from a very complex cell lysate, on one side of a non-siliceous surface (see, e.g., Claim 1) using an immobilization buffer while proteins and other types of contaminating molecules pass through to the other, opposing, "waste" side of the surface; then the immobilized nucleic acids are released with an elution agent in a highly purified form from the same side of the surface on which they were immobilized. Applicants' invention does not require lysis of cells by a cationic detergent as in step 1 of Schneider's "main" steps, above, nor does Applicants' claimed invention require the subsequent steps of extracting protein in the lysate solution by mixing in an organic solvent, followed by centrifugation of the aqueous-organic mixture to form an organic phase, protein clog, and aqueous phase from which the protein clog and organic phase are removed from the aqueous phase. *Indeed, Applicants' invention does not require any such organic phase extraction or centrifugation steps.* Note that, as with Ogawa above, Schneider must employ a separate step to eliminate contaminating protein: the protocol uses a subsequent filtration step that cannot discriminate between nucleic acids of interest and proteins or other types of molecules. Moreover, nowhere does Applicants' claimed process recite or depend on the formation of micellar cationic detergent-DNA complexes, the use of a filter of appropriate pore size (ultrafiltration, see, col. 2, lines 28-36, of Schneider) to retain such cationic detergent-DNA complexes from solution, and subsequent conversion of such a deliberately formed cationic detergent-DNA complex into a DNA low ionic strength salt solution. Furthermore, Schneider specifically teaches a preference for using a borosilicate glass filter for the ultrafiltration step (see, e.g., col. 2, lines 30-40, of Schneider). Applicants' claimed process specifically employs non-siliceous surfaces and membranes.

Applicants submit that the above explanation clearly shows that Schneider does not teach or suggest the claimed invention. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

Response to Rejections Under 35 USC § 102 and/or § 103: the Millipore Document

In the Office Action, the Examiner rejected Claims 1-3, 5, 24, 26, 28-29, 32, 39, 41, 50, and 58 as anticipated by or obvious over excerpts from a Millipore catalogue (1995) ("Millipore") obtained from the Millipore website (millipore.com/catalogue.nsf/docs/C7485; last visited 02/07/04). The Examiner also rejected Claims 1-3, 5, 9-22,24-32, 39, 41, 44-50, and 58-

64 as obvious over Millipore. For the reasons explained below, Applicants respectfully traverse the rejections.

Millipore describes the following microcentrifugation ("spin") products and their uses:

1. Removal of an enzyme from double-stranded DNA (dsDNA) samples using a Micropore-EZ enzyme remover device ("EZ device") is described as having "a high affinity for protein but not for dsDNA", but in particular can remove from solution certain enzymes that are active on or digest DNA but not others. See the lists of enzymes that are and are not removed by Micropore-EZ device in the Millipore document. Thus, the EZ device is an affinity chromatographic device that selectively binds a number of (but not all) enzymes used in various *in vitro* manipulations of dsDNA. For example, the EZ device may be used to separate an enzyme from dsDNA in a solution (i.e., provided the enzyme is known to be selectively bound by the affinity material in the EZ device). The EZ device is placed in a microcentrifuge vial, a solution of enzyme and dsDNA is applied to the top of the EZ device, the vial with the EZ device is spun briefly (e.g., 12,000 x *g* for 1-2 minutes) in a microcentrifuge, and the aqueous "filtrate" containing the dsDNA that passed through the enzyme-affinity material present in the EZ device is removed from the bottom of the vial.

2. Microcon Centrifugal Filter Devices ("Microcon") contain a low-binding YM regenerated cellulose ultrafiltration membrane. The Microcon devices are offered in a wide range of ultrafiltration cut-offs (size ranges) and may be used in concentrating, de-salting, and purifying microsamples of proteins *and* nucleic acids. Thus, Microcon devices are ultrafiltration devices that indiscriminately retain any molecule having a size within the fractionation range of the particular ultrafilter present in the device. To separate out certain sized molecules from a solution, the Microcon device with appropriate ultrafilter is placed in a microcentrifuge vial, the solution is applied to the top of the small ultrafilter in the Microcon device, and the vial with the Microcon device is then spun in a microcentrifuge for a recommended time depending on the size of the molecules in the solution. The Microcon device is then placed in an inverted position in a new microcentrifuge vial that is briefly spun to drive a small amount of liquid containing the concentrated molecules off the ultrafilter and into the new vial.

Millipore also describes the simultaneous use of the EZ device attached to a Microcon device to purify dsDNA that was subjected to an *in vitro* manipulation by an enzyme that is

known to be selectively bound by the affinity EZ device. In this process, the EZ protein affinity device is fixed to the top of a Microcon ultrafiltration device, and the connected devices placed in a microcentrifuge vial. A solution is applied to the top of the EZ device, and the vial is spun at a recommended time in a microcentrifuge. During the centrifugation, the solution passes through the EZ device where the enzyme is bound and then through the Microcon ultrafilter where the dsDNA should be retained. The devices are then disassembled, and the dsDNA retrieved from the Microcon ultrafilter as described above.

Applicants submit that Millipore clearly neither teaches nor suggests the claimed process for isolating nucleic acids. First, Applicants' claimed process is not restricted to separating certain enzymes from dsDNA, but is widely applicable to a variety of solutions, even to complex cell lysates comprising nucleic acids, proteins, lipids, carbohydrates, and other molecules of diverse types and sizes. Moreover, the method of Millipore first requires the step of separating dsDNA from only certain enzymes in a solution using a material with high affinity for certain enzymes, followed by the retention of dsDNA from the solution on a second (ultrafilter) surface. Such steps are simply not recited in Applicants' claimed process. Nowhere do Applicants' claims recite the use of a first surface or membrane that has a selective affinity for (certain) enzymes but not dsDNA and then employ an ultrafilter membrane to catch and retain the dsDNA based on its size. On the contrary, in Applicants' process, a solution containing nucleic acids of interest is applied to one side of a non-siliceous surface and the nucleic acids in the solution are preferentially immobilized on that side of the non-siliceous surface in the presence of an immobilization buffer *while contaminating proteins and other non-nucleic acid molecules pass through to the other (waste) side of the surface.* A person skilled in this art who reads Millipore is provided no teaching or suggestion of Applicants' process. Indeed, no centrifugation, size exclusion, or protein-affinity step is necessary for carrying out Applicants' claimed invention.

In view of the above comments, Applicants submit that it is clear that the steps and devices critical to processes in Millipore are clearly not recited in Applicants' claims. Accordingly, reconsideration and withdrawal of the rejections are respectfully requested.

In view of all of the above comments and amendments, Applicants submit that the claims are now in condition for allowance. Accordingly, entry of the amendments and allowance of the claims are respectfully solicited.

Respectfully submitted,

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OF CELL STRUCTURE AND FUNCTION

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the surface of the ribosome during protein biosynthesis (Chapter 33).

Further details of the structure and function of tRNAs are provided below and in Chapter 33 (page 929).

Ribosomal RNA

Ribosomal RNA (rRNA) constitutes up to 65 percent of the mass of ribosomes. It can be obtained from *E. coli* ribosomes in the form of linear, single-stranded molecules that appear in three characteristic forms, sedimenting at 23S, 16S, and 5S, respectively; these three forms differ in base ratios and sequences. In eukaryotic cells, which have larger ribosomes than prokaryotes, there are four types of rRNA; 5S, 7S, 18S, and 28S. Although rRNAs make up a large fraction of total cellular RNA, their function in ribosomes is not yet clear. A few of the bases in rRNAs are methylated.

Shorthand Representation of Nucleic Acid Backbones

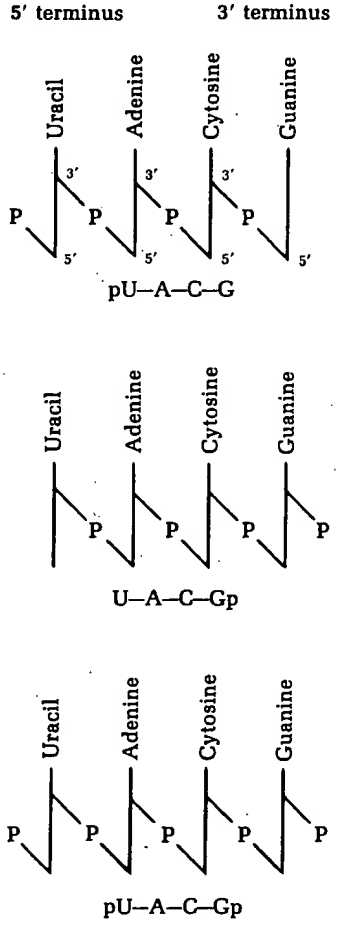
The covalent structure and base sequence of polynucleotide chains is often schematized as shown in Figure 12-14. These diagrams are also useful in indicating the specific bonds cleaved during chemical or enzymatic hydrolysis of nucleic acids, as we shall see below. In addition, an international convention is frequently used to indicate the nucleoside sequences of polynucleotides. The nucleosides of RNA are symbolized by A, U, G, and C; those of DNA by dA, dT, dG, and dC. The letter p designates a terminal phosphate group, a hyphen an internal phosphate group. When p appears to the left of a nucleoside symbol, the phosphate is esterified to the 5′ position; when it appears at the right of the nucleoside symbol, the phosphate is esterified to the 3′ position. Thus pA is adenosine 5′-phosphate and Ap is adenosine 3′-phosphate. Oligonucleotides (Greek *oligo*, "few") are conveniently symbolized as shown in the examples in Figure 12-14. To symbolize a DNA sequence the base symbols are prefixed by d, as in dA-T-G-Cp. The 3′ terminus of an oligonucleotide is that end at which the terminal nucleoside is attached by its 5′ carbon to the phosphoric group of the preceding nucleotide in the main chain.

Hydrolysis of Nucleic Acids by Acids and Bases

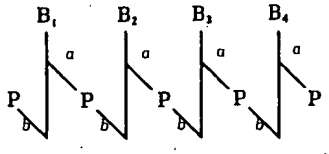
Gentle acid hydrolysis of DNA at pH 3.0 causes selective hydrolytic removal of all its purine bases without affecting the pyrimidine-deoxyribose bonds or the phosphodiester bonds of the backbone. The resulting DNA derivative, which is devoid of purine bases, is called an *apurinic acid*. Selective removal of the pyrimidine bases, accomplished by somewhat different chemical conditions, produces *apyrimidinic acid*.

DNA is not hydrolyzed by dilute alkali, whereas RNA is because of the 2′-hydroxyl groups it contains. Dilute sodium hydroxide produces from RNA a mixture of nucleoside

Figure 12-14
Schematic representation and shorthand notation of polynucleotide structure. The vertical lines represent the pentose backbone, the numbers 3′ and 5′ the carbon atoms of the pentose, and P the phosphoric group. The diagrams always show the 3′,5′ phosphodiester linkage going from left to right. The shorthand notation for oligodeoxyribonucleotides may include the prefix d, if needed.



The a (3′) and b (5′) linkages (color) of the phosphodiester internucleotide bonds.



2′- and 3′-phosphates (Figure 12-10). Cyclic 2′,3′-monophosphates are the first products of the action of alkali on RNA and are obligatory intermediates; they are then further hydrolyzed by the alkali, which attacks either one of the two P—O—C linkages to yield a mixture of 2′- and 3′-nucleoside monophosphates (Figure 12-10). The discovery of this mechanism explains why DNA is not hydrolyzed by base: DNA has no 2′-hydroxyl groups and therefore cannot form the necessary 2′,3′-cyclic monophosphate intermediates. Nucleoside 2′,3′-cyclic monophosphates are also intermediates in the action of some ribonucleases (see below).

Selective hydrolytic cleavage of polynucleotides by enzymatic or chemical methods is used in determining the base sequence of nucleic acids, which in principle is approached with the same logic as that employed in the sequence analysis of proteins (page 98).

Enzymatic Hydrolysis of Nucleic Acids

Nucleic acids ingested by animals undergo enzymatic hydrolysis in the intestine by nucleases secreted by the pancreas. These and other enzymes capable of hydrolyzing nucleic acids are important tools in analyzing nucleotide sequence. The phosphodiester bridges of DNA and RNA are attacked by two classes of enzymes, designated a and b (or 3′ and 5′) in Figure 12-14 and Table 12-4, depending on which side of the phosphodiester bridge is attacked. The a, or 3′, enzymes specifically hydrolyze the ester linkage between the 3′ carbon and the phosphoric group, and the b, or 5′, enzymes hydrolyze the ester linkage between the phosphoric group and the 5′ carbon of the phosphodiester bridge (Figure 12-14). The best known of the class a enzymes is a phosphodiesterase from the venom of the rattlesnake or Russell's viper, which hydrolyzes all the 3′ bonds in either RNA or

Table 12-4 Specificity of some enzymes acting on nucleic acids

Enzyme	*Nucleic acid*	*Specificity*
	Class a (3′) nucleases	
Exonuclease		
Snake-venom phosphodiesterase	DNA and RNA	Starts from 3′ end
Endonuclease		
Deoxyribonuclease I	DNA	Some 3′ linkages
	Class b (5′) nucleases	
Exonuclease		
Spleen phosphodiesterase	DNA and RNA	Starts from 5′ end
Endonucleases		
Deoxyribonuclease II	DNA	Some 5′ linkages
Ribonuclease I (pancreas)	RNA	5′ linkages in which the 3′ linkage is to pyrimidine nucleotide
Ribonuclease T_1 (mold)	RNA	5′ linkages in which the 3′ linkage is to a guanine nucleotide